The 'dynamics' in the thermodynamics of binding

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Assumptions of restricted flexibility upon binding conflict with emerging data showing that motion can increase, decrease or stay the same within molecular complexes. Now, calculations of entropic contributions from dynamics at specific positions in a complex suggest that increases in motion can dominate the free energy of association in certain cases.

Protein recognition is a critical component of the regulation and function of a majority of biological processes. Whether two molecules will bind is determined by the free energy change (ΔG) of the interaction, composed of both enthalpic and entropic terms. X-ray crystallographic and NMR solution structures of proteins in complex with their targets enable a reasonably detailed description of the static interactions giving rise to enthalpic contributions to this binding free energy. However, the entropic component is more difficult to address. Over the past few years, NMR relaxation studies have been applied to study proteins in both free and complexed states, and insights into this more elusive term are beginning to emerge. In an intriguing paper discussing the significance of conformational entropy in binding free energy on page 1118 of this issue of *Nature Structural Biology*[1], Zídek *et al.* show that the fast timescale motions of the backbone of the mouse major urinary binding protein I increase upon binding its target, the small hydrophobic pheromone 2-*sec*-butyl-4,5-dihydrothiazole. The small increase in motion observed for nearly all residues of the protein correspond to a significant increase in backbone conformational entropy with quantitative estimates suggesting that this entropy term plays a dominant role in favoring binding.

These results are surprising given the conventional assumption that binding reduces motion[2]. The 'induced fit' model of binding provides an intuitive picture that reinforces the idea of 'freezing out' multiple possible conformations upon binding. In addition, the packed core of a protein is assumed to be quite rigid, and high affinity interactions are thought to generate well-packed interfaces similar to these protein cores. While such views are prevalent, little experimental data has been available to either support or refute them.

Nuclear magnetic resonance spectroscopy is sensitive to motions over a wide range of timescales and can provide site-specific dynamic information. During an NMR relaxation experiment, non-equilibrium magnetization is created and the decay of the magnetization back to equilibrium is observed. One mechanism of this decay is the presence of a fluctuating magnetic field. Since NMR-active nuclei can be viewed as small bar magnets, atomic motion gives rise to magnetic field fluctuations that relax nearby nuclei. Fluctuations of covalently attached nuclear 'bar magnets' can have a very significant effect. Thus, analysis of ^{15}N relaxation data provides information on the backbone motion of the ^{15}N-^{1}H amide bond vector. Side chain bond vectors can also be probed by ^{13}C or ^{2}H relaxation experiments.

These experiments have been applied to both free and complexed states of proteins, enabling comparison of their backbone and side chain dynamics[3–7]. A common method for extracting motional information from relaxation data, the 'Lipari-Szabo model-free' approach[8,9], allows the calculation of an overall correlation time, τ_m, a correlation time for internal motions, τ_e, and an order parameter, S^2, based on the measurement of three different types of relaxation rates. The overall correlation time, which is the same for all residues in a protein, is the time constant for rotational diffusion. An order parameter is a measure of the amplitude of the internal motions. Order parameters vary from $S^2 = 1$, for a bond vector having no internal motion, to $S^2 = 0$, for a bond vector rapidly sampling multiple orientations, independent of the overall motion of the protein, leading to complex averaging.

As more NMR relaxation data have become available for both backbone and side chain positions, it is possible to test the assumptions of rigidity in protein cores and interfaces between two proteins that bind. Molecular dynamics simulations of barstar highlight significant side chain mobility within the hydrophobic core of the protein[10], which is in good agreement with emerging NMR methyl relaxation data[11] but is surprising in light of prevalent images of proteins. For binding interfaces, relaxation data have demonstrated that certain residues in apo forms are more flexible than in the ligand-bound states, providing partial support for the view that binding leads to restricted conformational flexibility.

For example, the binding of Ca^{2+} by calbindin has been studied using backbone ^{15}N relaxation. The results demonstrate that order parameters in one binding loop remain unchanged after ligand binding but those of the other binding loop increase significantly[12]. In another example, the backbone order parameters for a number of residues in the phosphotyrosine binding domain of insulin receptor substrate-1 that interact with a ligand peptide are high in both free and complexed states, while those for residues in other regions increase upon ligand binding — that is, they become less mobile. Of interest is the observation that certain residues not making any direct contacts with the peptide ligand also become less dynamic upon binding[13].

However, evidence is mounting that binding does not always lead to motional restriction. Studies of the backbone dynamics of the C-terminal domain of topoisomerase I demonstrate that while many residues show increased order parameters, a few residues become more mobile in the presence of DNA[14]. Similar results were observed for the enzyme 4-oxalocrotonate tautomerase in the presence of its inhibitor[15]. In addition, certain positions on the binding surface can be highly dynamic in both free and complexed states, based on studies of an SH2 domain–phosphopeptide interaction[16].

Order parameters can provide an important conceptual context for addressing the thermodynamics of binding, since a decrease in motion implies less conformational sampling and thus lower entropy. However, there is still no rigorous method for obtaining a quantitative measure of the difference in conformational entropy due to changes in the motion of residues upon protein binding. Important steps in this direction have been taken, though, with recent attempts to calculate thermodynamic parameters from NMR relaxation data[17–19].

The approach used to calculate conformational entropy from order parameters utilized by Zídek *et al.*[1] is based on a method developed by Yang and Kay[18]. In this method, an entropy-order parameter profile derived from a molecular dynamics simulation has been fit to an analytical expression that assumes the bond vector motion can be described as diffusion within a cone. Thus, for single sites within a protein, it is possible to directly relate conformational entropy to the nanosecond-picosecond timescale motion of bond vector fluctuations. However, it remains unclear how to determine the overall entropy of a protein from NMR relaxation data because (i) the motion at each site is not independent, (ii) it is not possible to measure order parameters at all bond vectors in a protein, and (iii) order parameters do not reflect motion of timescales outside of the nanosecond-picosecond timescale probed by NMR relaxation studies. Nonetheless, this approach is being applied in an increasing number of cases[15,20–24] to provide a semi-quantitative thermodynamic interpretation of the NMR relaxation data.

In particular, the change in conformational entropy upon folding or binding is of significant interest and entropy calculations have been applied to examples of both. From a recent backbone and methyl relaxation study of the regulatory domain of troponin C[23], there is a 1 kcal mol^{-1} difference in conformational entropy loss upon Ca^{2+} binding between the two calcium sites. This is consistent with the difference in measured affinities of these sites for calcium. Thus, it was suggested that entropy plays a role in 'fine tuning' these calcium affinities. In addition, methyl dynamics of residues exposed upon binding were found to be restricted in the apo state compared to the likely more flexible Ca^{2+}-bound state, demonstrating another potential role for increasing entropy in the protein to compensate for entropy loss upon exposure of hydrophobic groups.

In a study of the basic leucine zipper of GCN4 binding to DNA, a conformational entropy change of -0.6 kJ mol^{-1} was calculated by adding the contributions from each backbone position[22] using an approach similar to that described above with a slightly different analytical expression relating order parameters to entropy[17]. The resulting value compares favorably with a conformational entropy change of -1.2 kJ mol^{-1} estimated from calorimetry, which includes side chain contributions that are thought to account for 55–60% of the overall conformational entropy. Thus, taking into consideration the uncertainties in the estimates and the assumption of independent motion of backbone groups, the quantitative agreement is quite impressive.

Among the reported investigations of protein dynamics and binding, the paper by Zídek *et al.*[1] is important because it contributes additional evidence that motions of a protein can increase upon binding its target. This is reflected by the general decrease in order parameters throughout the protein after substrate binding. A particularly exciting aspect of the work is that it might provide a general explanation for the binding of small hydrophobic ligands. The enthalpic interactions upon binding of such ligands are likely to be small since they are related to the surface area[25]. However, since the loss of rotational and translational entropy upon binding does not scale with the size of the ligand, compensating energetic components for binding must be found to allow an understanding of how binding occurs. For molecules such as the pheromone 2-*sec*-butyl-4,5-dihydrothiazole, electrostatic interactions do not play a large role. The authors suggest that the observed small yet significant increases in motion throughout the protein provide a compensating entropic energy component necessary to drive binding. While some may argue that small changes in order parameter (<0.1 in this study) are difficult to interpret, as they may vary with different motional models used to fit the data, the changes occurring at many sites throughout the protein make a convincing case that a subtle increase in dynamic motion could be used to increase entropy without significantly perturbing the molecular structure or local stability of the protein.

The importance of understanding site-specific thermodynamic contributions to binding is beginning to be recognized. Theoretical and calorimetric approaches as well as alanine scanning mutagenesis of interfacial residues have been used to probe thermodynamic 'hot spots' for binding in macromolecular complexes[26–28]. The increased power of NMR spectroscopy to characterize motion at individual positions provides a new tool with the potential to delineate site-specific entropic contributions. Proteins have evolved a variety of ways to compensate for entropy loss upon binding — only through further investigations like that of Zídek *et al.*[1] will the role of dynamics in driving molecular interactions become clear.

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